

FIGURE 1: Expression of a 43 kDa protein corresponding to mature Der p1 in fusion with the prepeptide MF-alpha of *Pichia pastoris* (construct pNIV4811) in yeast cells. The culture supernatants from various *Pichia pastoris* clones incubated in the absence or presence of methanol (methanol induction for 1 to 5 days indicated on the x axis) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody.

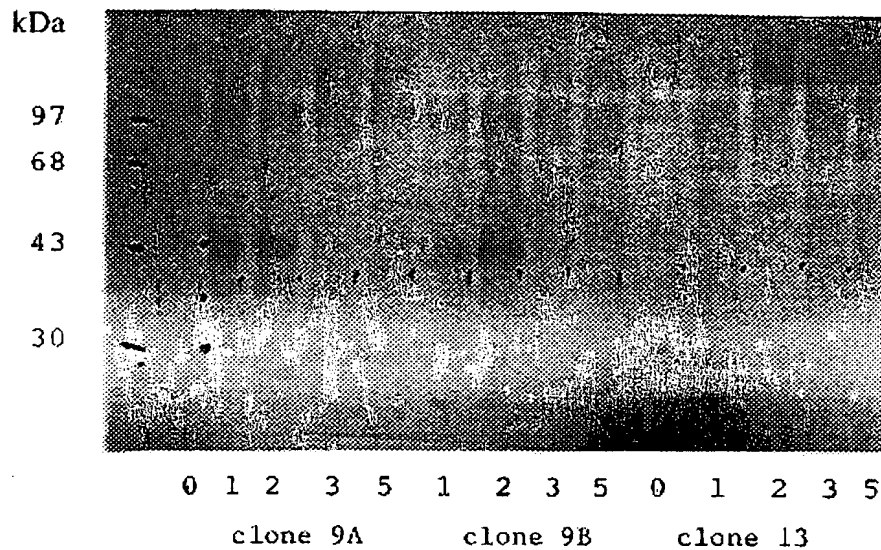


Figure 1

FIGURE 2: Expression of mature Der p1 (30 kDa) in fusion with the prepeptide of *Pichia pastoris* MF-alpha (construct pNIV4817) in yeast cells. The culture supernatants from *Pichia pastoris* cells incubated in the absence (J0) or presence of methanol for 1 day (J1) have been concentrated 50 times and, then, analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Arrows indicate the mature Der p1 doublet at about 30 kDa

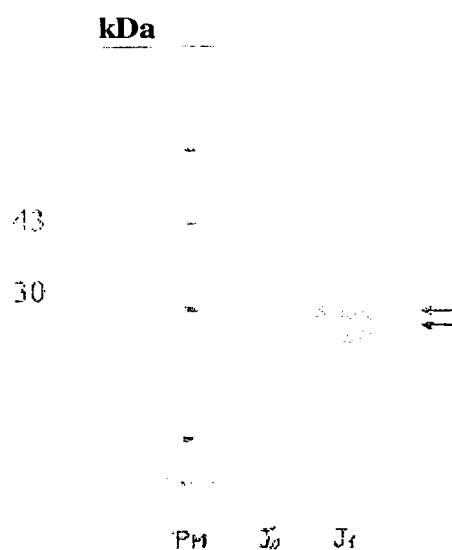


Figure 2

FIGURE 3: Expression of Der p1 in fusion with its propeptide (construct pNIV4812) in CHO-K1 cells. The cell extracts from different clones of CHO-K1 cells transfected with pNIV4812 (lanes 3-8) or transfected with the vector pEE14 alone as negative controls (lane 1 & 2) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. The arrow indicates the mature Der p1 protein.

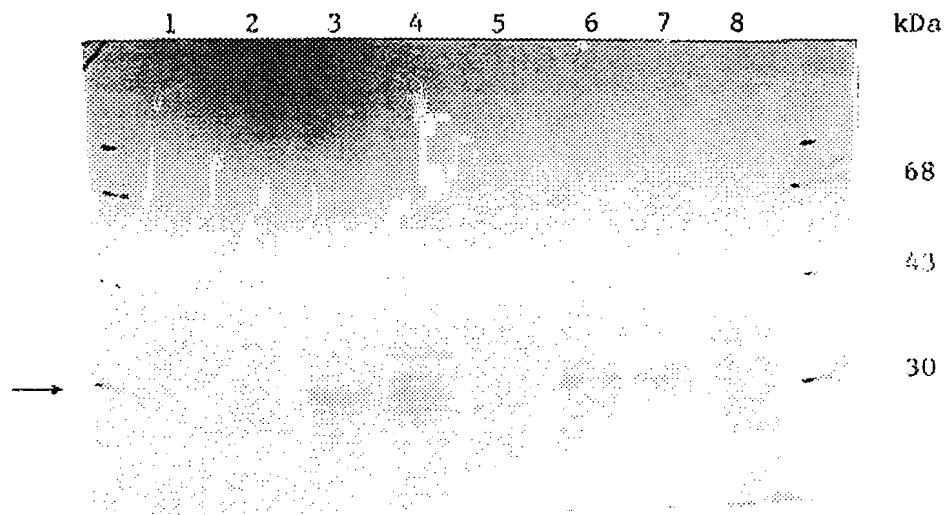


Figure 3

FIGURE 4: Expression of Der p1 in fusion with its propeptide (construct pNIV4840) in drosophila cells S2 (Invitrogen). The cell extracts of different clones of CHO-K1 cells transfected with pNIV4840 (lanes 1 & 4) or transfected with the inducible vector pMT/V5-His alone as negative controls (lanes 2,3,5, & 6) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. The induction has been carried out for 22 hours (1-3) and 28 hours (4-6).

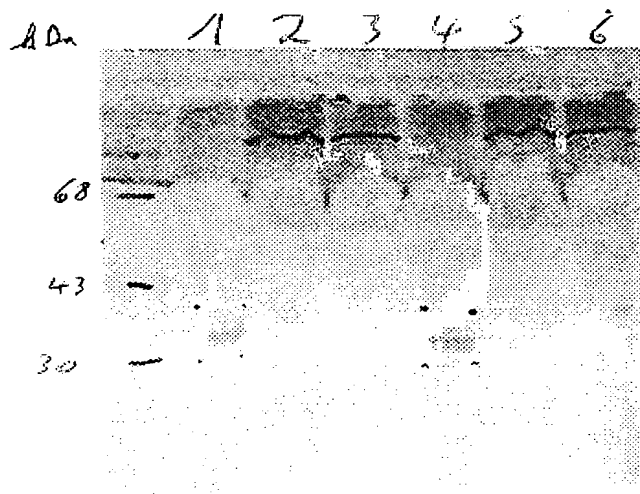


Figure 4

FIGURE 5: Expression of non-cleavable, non-activable Der p1 mutant in fusion with its pro-peptide (construct pNIV4842) in drosophila cells S2 (Invitrogen). The cell supernatants from transiently transfected S2 cells with pNIV4842 (lanes 1-4) or transfected with the inducible vector pMT/V5-His alone as negative control (lanes 5) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Lanes 1 to 4 correspond to 1, 4, 5, and 6 days of induction, respectively. Arrows indicate the pro Der p1 doublet at about 36 kDa.

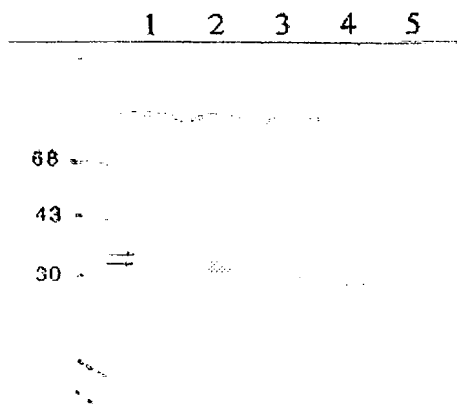


Figure 5

FIGURE 6: Expression of non-active Der p1 mutant in fusion with its propeptide (construct pNIV4843) in drosophila cells S2. The cell supernatants from transiently transfected S2 cells with pNIV4843 (lanes 6-9) or transfected with the inducible vector pMT/V5-His alone as negative control (lanes 5) have been analyzed by SDS-PAGE and immunoblot analysis using an anti-Der p1 peptide (117-133) polyclonal antibody. Lanes 6 to 9 correspond to 1, 4, 5, and 6 days of induction, respectively. Arrows indicate the mature Der p1 doublet at about 36 kDa

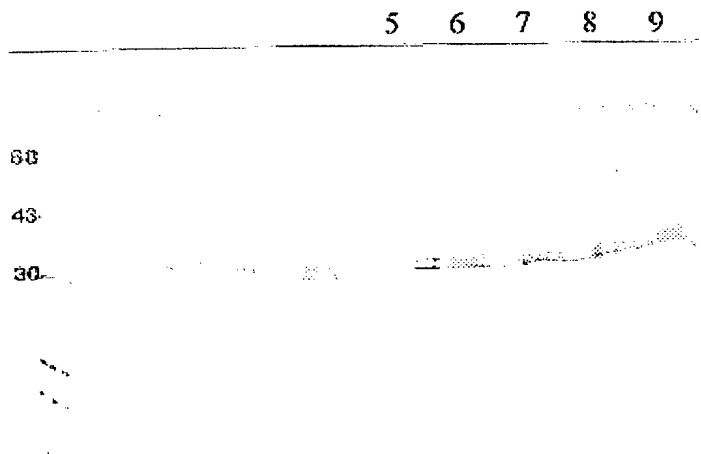
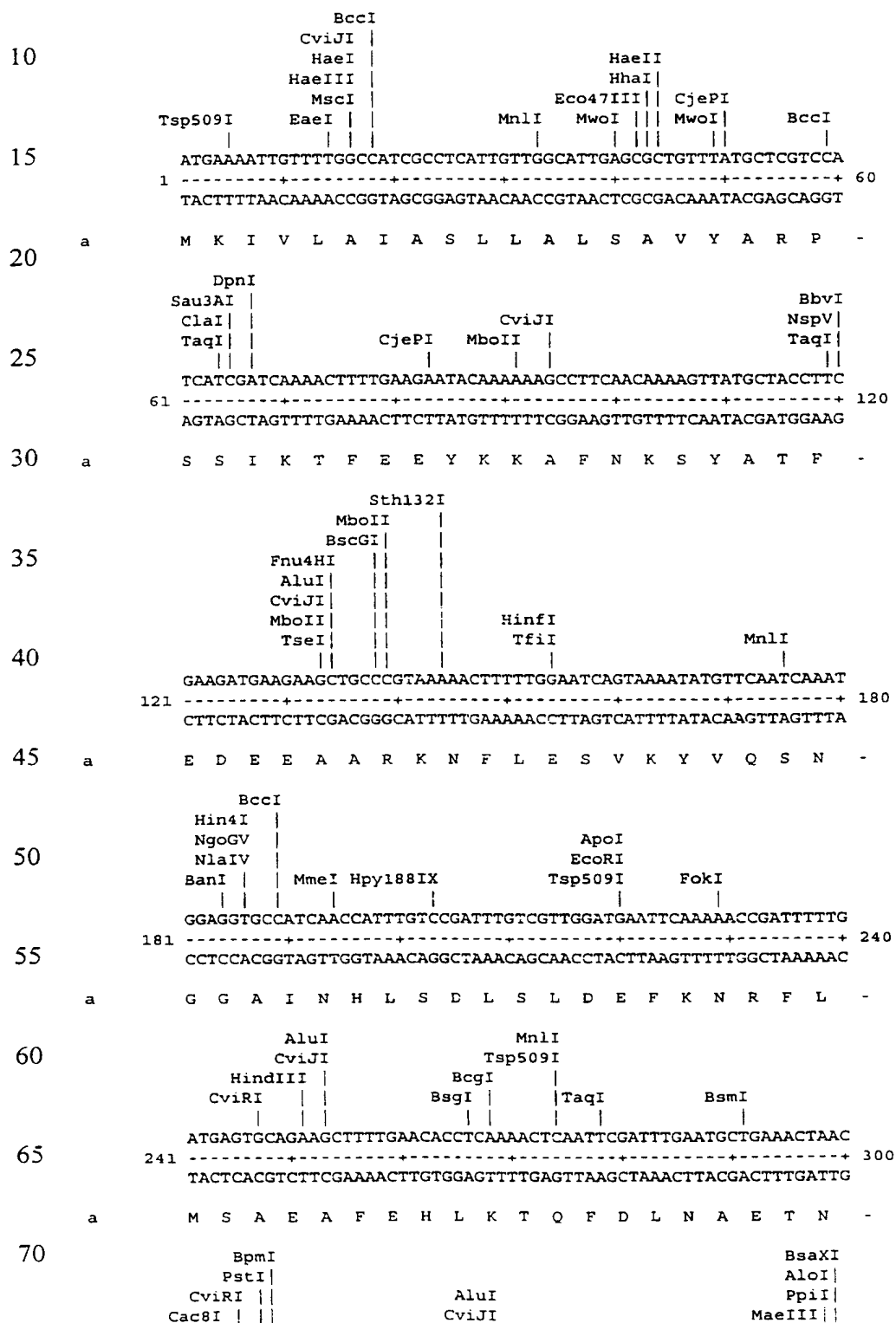


Figure 6

ID DP11695 standard; RNA; INV; 1099 BP.



SfcI | | | MspAII | ClaI | CjePI | TaaI | |
 BcgI | | | CjePI | PvuII | TaqI | MwoI | Tsp45I | |
 | | | | | | | | | | | | | |
 5 GCCTGCAGTATCAATGGAATGCTCCAGCTGAAATCGATTGCGACAAATGCGAACTGTC 360
 301 -----+-----+-----+-----+-----+-----+-----+
 CGGACGTCATAGTTACCTTTACGAGGTCGACTTTAGCTAAACGCTGTTTACGCTTGACAG
 a A C S I N G N A P A E I D L R Q M R T V -
 10
 CviRI | | | | | | | | | | | | | |
 MnlI | | | | | | | | | | | | | |
 MslI | | | CviJI | DrdII | | CviJI | | | BsbI | | |
 15 ACTCCCATTCGTATGCAAGGAGGCTGTGGTTTCATGTTGGGCTTCTCTGGTGTGCGCA 420
 361 -----+-----+-----+-----+-----+-----+-----+
 TGAGGGTAAGCATACGTTCTCCGACACCAAGTACAACCCGAAAGAGACCACAACGGCGT
 a T P I R M Q G G C G S C W A F S G V A A -
 20
 DpnI | | | | | | | | | | | | | |
 HinfI | AluI | CviJI | | | BstYI | | | | | | | |
 TfiI | CviJI | MwoI | | | TaaI | | | Sau3AI | | | AlwI | Tsp509I | |
 25 ACTGAATCAGCTTATTTGGCTTACCCTAATCAATCATTGGATCTTGCTGAACAAGAATTA 480
 421 -----+-----+-----+-----+-----+-----+-----+
 TGACTTAGTCGAATAAACCGAATGGCATTAGTTAGTAACCTAGAACGACTTGTCTTAAT
 a T E S A Y L A Y R N Q S L D L A E Q E L -
 30
 BsaAI | | | | | | | | | | | | | |
 FokI | | | | | | | | | | | | | |
 PmlI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 35 TaqI | | | BsbI | | | TaaI | | | NlaIII | | | CjeI | | | HphI | | |
 | | | | | | | | | | | | | |
 GTCGATTGTGCTTCCCAACACGGTGTGTCATGGTGATACCATCCACGTGGTATTGAATAC 540
 481 -----+-----+-----+-----+-----+-----+-----+
 CAGCTAACACGAAGGTTGTGCCAACAGTACCCTATGGTAAGGTGCACCATACTTATG
 a V D C A S Q H G C H G D T I P R G I E Y -
 40
 AluI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 45 CjeI | BstXI | | | MmeI | | | TaqI | | | CviRI | | | BssSI | | |
 | | | | | | | | | | | | | |
 ATCCAACATAATGGTGTGCTCCAGAAAGCTACTATCGATACGTTGCACGAGAACAATCA 600
 541 -----+-----+-----+-----+-----+-----+-----+
 TAGGTTGTATTACCACAGCAGGTTCTTTTCGATGATAGCTATGCAACGTGCTCTTGTAGT
 a I Q H N G V V Q E S Y Y R Y V A R E Q S -
 50
 AclI | | | | | | | | | | | | | |
 MaeII | | | | | | | | | | | | | |
 55 NlaIII | | | CviRI | | | ApoI | | | Tsp509I | | | CjeI | | |
 | | | | | | | | | | | | | |
 TGCCGACGACCAATGCACAACGTTTCGGTATCTCAAATATTGCCAAATTTACCCACCA 660
 601 -----+-----+-----+-----+-----+-----+-----+
 ACGGCTGCTGGTTTACGTGTTGCAAAGCCATAGAGTTTGATAACGGTTTAAATGGGTGGT
 a C R R P N A Q R F G I S N Y C Q I Y P P -
 60
 AluI | | | | | | | | | | | | | |
 CviJI | | | | | | | | | | | | | |
 65 CjeI | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 Hpy178III | | | | | | | | | | | | | |
 HaeII | | | | | | | | | | | | | |
 HhaI | | | | | | | | | | | | | |
 70 ApoI | | | | | | | | | | | | | |
 Tsp509I | | | | | | | | | | | | | |
 | | | | | | | | | | | | | |
 AATGTAACAAAATTGCTGAAGCTTTGGCTCAAACCCACAGCGCTATTGCCGTCATTATT 720
 661 -----+-----+-----+-----+-----+-----+-----+
 TTACATTTGTTTTAAGCACTTCGAAACCGAGTTTGGGTGTCGCGATAACGGCAGTAATAA

09554960, 051900

a N V N K I R E A L A Q T H S A I A V I I -

5 CviJI
HaeIII
BccI
EaeI
GdiII

10 SfaNI BsmI HgaI MslI Thai

GGCATCAAAGATTTAGACGCATTCCGTCATTATGATGGCCGAACAATCATTCAACGCGAT
721 -----+----- 780
CCGTAGTTTCTAAATCTGCGTAAGGCAGTAATACTACCGGCTTGTTAGTAAGTTGCGCTA

15 a G I K D L D A F R H Y D G R T I I Q R D -

BstEII MaeIII
HincII MaeIII TaaI DraIII

20 AATGGTTACCAACCAAACTATCAGCTGTCAACATTGTTGGTTACAGTAACGCACAAGGT
781 -----+----- 840
TTACCAATGGTTGGTTTGATAGTGCACAGTTGTAACAACCAATGTCATTGCGTGTCCA

25 a N G Y Q P N Y H A V N I V G Y S N A Q G -

CjeI
TaaI
BciVI
CjePI
AlwI
RsaI
SunI
DpnI
TaqI
Sau3AI
HgiEII
MunI
Tsp509I
MaeIII
CjeI
TaaI
CjePI
HphI
BbvI

30
35

GTCGATTATTGGATCGTACGAAACAGTTGGGATACCAATTGGGGTGATAATGGTTACGGT
841 -----+----- 900
CAGCTAATAACCTAGCATGCTTTGTCAACCCTATGGTTAACCCCACTATTACCAATGCCA

40 a V D Y W I V R N S W D T N W G D N G Y G -

Fnu4HI ClaI Bsp24I
TseI TaqI CjePI MboII
CjeI NdeI

45 TATTTTGCTGCCAACATCGATTGATGATGATTGAAGAATATCCATATGTTGTCATTCTC
901 -----+----- 960
ATAAAACGACGGTTGTAGCTAACTACTACTAATTCTTATAGGTATACAACAGTAAGAG

50 a Y F A A N I D L M M I E E Y P Y V V I L -

TAA
961 --- 963
ATT

55

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